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(54) Title: CANINE GROWTH HORMONE SECRETAGOGUE RECEPTOR

(57) Abstract

A novel canine cDNA sequence that encodes the canine growth hormone secretagogue receptor (GHSR) protein is provided. Also provided is canine GHSR protein encoded by the novel cDNA sequence. Methods of expressing canine GHSR protein in recombinant systems are provided. Also provided are methods for identifying agonists and antagonists of the canine GHSR.

TITLE OF THE INVENTION
CANINE GROWTH HORMONE SECRETAGOGUE RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS
5   Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D
               Not applicable.

10   REFERENCE TO MICROFICHE APPENDIX
               Not applicable.

FIELD OF THE INVENTION
               The present invention is directed to a novel canine DNA sequence
15   encoding the canine growth hormone secretagogue receptor as well as to the protein
encoded by that DNA.

BACKGROUND OF THE INVENTION
               Growth hormone (GH) is an anabolic hormone capable of promoting
20   linear growth, weight gain and whole body nitrogen retention in mammals. GH is
thought to be released primarily from the somatotroph cells of the anterior pituitary
under the coordinate regulation of two hypothalamic hormones, growth hormone
releasing factor (GHRF or GRF) and somatostatin. Both GHRF stimulation and
somatostatin inhibition of the release of GH occurs by the specific engagement of
25   receptors on the cell membrane of the somatotroph.
               GH release is also stimulated by a group of short peptides known as
growth hormone releasing peptides (GHRPs) or as growth hormone secretagogues.
Among the GHRPs are GHRP-2 (hexarelin) and GHRP-6, which are described, for
example, in U.S. Patent No. 4,411,890, PCT Patent Pub. No. WO 89/07110, PCT
30   Patent Pub. No. WO 89/07111, PCT Patent Pub. No. WO 93/04081, and *J.
Endocrinol Invest.*, *15* (Suppl 4), 45 (1992). GHRPs function by selectively binding
to distinct somatotroph cell membrane receptor(s), the growth hormone secretagogue
receptor(s) (GHSRs). A medicinal chemical approach has resulted in the design of
several classes of orally-active, low molecular weight, non-peptidyl compounds which
35   bind specifically to human GHSRs and result in the pulsatile release of GH. Such

compounds possessing growth hormone secretagogue activity are disclosed, for example, in the following: U.S. Patent No. 3,239,345; U.S. Patent No. 4,036,979; U.S. Patent No. 4,411,890; U.S. Patent No. 5,206,235; and U.S. Patent No. 5,283,241.

5   The use of orally-active agents which engage GHSRs and thus stimulate the pulsatile release of GH has been a significant advance in the treatment of growth hormone deficiency in children and adults and provides substantial benefit under circumstances where the anabolic effects of GH can be exploited clinically (e.g., post-hip fracture rehabilitation, the frail elderly, and in post-operative recovery patients). Other uses for such agents are being discovered from time to time. For

10   example, Copinschi et al., 1997, Neuroendocrinol. 66:278-286 indicates that treatment with the non-peptidyl growth hormone secretagogue MK-677 improves sleep quality.

Provision of GHSRs from non-human species would allow for the development of a new set of agents that could be used as veterinary pharmaceuticals

15   in animal disease states analagous to those described above for humans.

SUMMARY OF THE INVENTION

The present invention is directed to a novel canine DNA that encodes the canine growth hormone secretagogue receptor (GHSR). The DNA encoding

20   canine GHSR is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. Also provided is a canine GHSR protein encoded by the novel DNA sequence. The canine GHSR protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:2. Methods of expressing the canine GHSR protein in recombinant systems and of identifying

25   agonists and antagonists of the canine GHSR are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a cDNA sequence encoding canine growth hormone secretagogue receptor (GHSR) (SEQ.ID.NO.:1). The sequence shown begins with the

30   ATG start codon and ends with the TGA stop codon.

Figure 2 shows the amino acid sequence of canine GHSR (SEQ.ID.NO.:2).

Figure 3 shows a comparison of the amino acid sequences of the human (SEQ.ID.NO.:5), rat (SEQ.ID.NO.:6), swine (SEQ.ID.NO.:7), and canine

35   (SEQ.ID.NO.:2) GHSRs.

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DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably

5   95%, more preferably 99%, and even more preferably 99.9%, free of other proteins.
Thus, a canine GHSR protein preparation that is substantially free from other proteins
will contain, as a percent of its total protein, no more than 10%, preferably no more
than 5%, more preferably no more than 1%, and even more preferably no more than
0.1%, of non-canine GHSR proteins. Whether a given canine GHSR protein

10   preparation is substantially free from other proteins can be determined by such
conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate
polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection
methods, *e.g.*, silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least 90%,

15   preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other
nucleic acids. Thus, a canine GHSR DNA preparation that is substantially free from
other nucleic acids will contain, as a percent of its total nucleic acid, no more than
10%, preferably no more than 5%, more preferably no more than 1%, and even more
preferably no more than 0.1%, of non-canine GHSR nucleic acids. Whether a given

20   canine GHSR DNA preparation is substantially free from other nucleic acids can be
determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*,
agarose gel electrophoresis combined with appropriate staining methods, *e.g.*,
ethidium bromide staining, or by sequencing.

A polypeptide has "substantially the same biological activity" as canine

25   GHSR protein if that polypeptide has a K_d for a ligand of canine GHSR protein such
as, *e.g.*, MK-677, GHRP-6, L-692,429, that is no more than 5-fold greater than the K_d
of canine GHSR protein having the amino acid sequence of SEQ.ID.NO.:2 for the
same ligand.

A "conservative amino acid substitution" refers to the replacement of

30   one amino acid residue by another, chemically similar, amino acid residue. Examples
of such conservative substitutions are: substitution of one hydrophobic residue
(isoleucine, leucine, valine, or methionine) for another; substitution of one polar
residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic
acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine,

35   or phenylalanine) for another.

The present invention relates to the identification and cloning of a cDNA encoding the canine GHSR. The present invention includes DNA molecules substantially free from other nucleic acids comprising SEQ.ID.NO.:1.

The novel DNA sequences of the present invention encoding canine
5   GHSR, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which cDNA encoding canine GHSR is not naturally linked, to form "recombinant DNA molecules" encoding canine GHSR. Such other sequences can include DNA sequences that control transcription or translation such as, *e.g.*, translation initiation sequences, promoters for RNA polymerase II, transcription or
10   translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast
15   artificial chromosomes. Accordingly, the present invention includes recombinant DNA molecules comprising SEQ.ID.NO.:1.

Included in the present invention are DNA sequences that hybridize to SEQ.ID.NO.:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters
20   containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1%
25   SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at
30   65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, <u>Molecular Cloning: A Laboratory Manual</u>, second edition, Cold Spring Harbor

Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the canine GHSR protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:1, but still encodes the same canine GHSR protein as SEQ.ID.NO.:1. Such synthetic DNAs are intended to be within the scope of the present invention.

Other DNA sequences included in the present invention are those DNA sequences that are identical to SEQ.ID.NO.:1 except that the last three nucleotides (TGA, the stop codon) differ. In particular, the stop codon of SEQ.ID.NO.:1 can be replaced by other stop codons (TAA or TAG).

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding canine GHSR protein. Such recombinant host cells can be cultured under suitable conditions to produce canine GHSR protein. An expression vector containing DNA encoding canine GHSR protein can be used for expression of canine GHSR protein in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of canine GHSR protein and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

A variety of mammalian expression vectors can be used to express recombinant canine GHSR in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110),

pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, canine GHSR protein can be purified by conventional techniques to a level that is substantially free from other proteins.

5   The present invention includes canine GHSR protein substantially free from other proteins. The amino acid sequence of the full-length canine GHSR protein is shown in Figure 2 as SEQ.ID.NO.:2. Thus, the present invention includes canine GHSR protein substantially free from other proteins having the amino acid sequence of SEQ.ID.NO.:2.

10   The amino acid sequence of the canine GHSR protein (SEQ.ID.NO.:2) indicates that the canine GHSR protein contains many of the characteristic features of G-protein coupled receptors (GPCRs):

     (a)   seven transmembrane domains;
     (b)   three intracellular loops;
15   (c)   three extracellular loops; and
     (d)   the GPCR triplet signature sequence.

As with many proteins, it is possible to modify many of the amino acids of canine GHSR and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified canine GHSR proteins
20   which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as canine GHSR. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., Molecular Biology of the Gene, Watson et al., 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells,
25   1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as canine GHSR protein. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:2 wherein the
30   polypeptides still retain substantially the same biological activity as canine GHSR protein. In particular, the present invention includes canine GHSR polypeptides containing two or more amino acid substitutions where the substitutions do not occur in positions where all four of the human, rat, swine, and canine GHSRs share the same amino acid (see Figure 3) and where the protein has substantially the same
35   biological activity as canine GHSR having the amino acid sequence SEQ.ID.NO.:2.

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In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand binding domain.

5   The canine GHSR proteins of the present invention may contain post-translational modifications, *e.g.*, covalently linked carbohydrate, phosphorylated tyrosine.

The present invention also includes chimeric canine GHSR proteins. Chimeric canine GHSR proteins consist of a contiguous polypeptide sequence of at

10   least a portion of a canine GHSR protein fused to a polypeptide sequence of a non-canine GHSR protein. For example, the N-terminal domain and seven transmembrane spanning domains of canine GHSR protein fused at the C-terminus in frame to a G protein would be a chimeric canine GHSR protein.

The present invention also includes canine GHSR proteins that are in

15   the form of multimeric structures, *e.g.*, dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227, 200-204; Romano *et al.*, 1996, J. Biol. Chem. 271, 28612-28616).

The present invention also includes isolated forms of canine GHSR

20   proteins. By "isolated canine GHSR protein" is meant canine GHSR protein that has been isolated from a natural source. Use of the term "isolated" indicates that canine GHSR protein has been removed from its normal cellular environment. Thus, an isolated canine GHSR protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does

25   not imply that an isolated canine GHSR protein is the only protein present. but instead means that an isolated canine GHSR protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the canine GHSR protein. Thus, a canine GHSR protein that is expressed through recombinant means in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without

30   human intervention) express it is an "isolated canine GHSR protein."

A cDNA fragment encoding full-length canine GHSR protein can be isolated from a canine cDNA library constructed from poly (A)+ mRNA frm hypothalamus or pituitary gland by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the

cDNA sequence for canine GHSR shown in Figure 1 as SEQ.ID.NO.:1. Suitable
primer pairs would be, *e.g.*:

> ATGCGGAACGCGACGGCC (SEQ.ID.NO.:3) and
> TCATGTATTAATACTTAG (SEQ.ID.NO.:4).

5   The above-described primers will allow for the amplification of the complete cDNA.
These primers are meant to be illustrative. Other suitable primers will be evident to
those skilled in the art.

PCR reactions can be carried out with a variety of thermostable
enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase.
10   For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM
$MgCl_2$, 200 μM for each dNTP, 50 mM KCl, 0.2 μM for each primer, 10 ng of DNA
template, 0.05 units/μl of AmpliTaq. The reactions are heated at 95°C for 3 minutes
and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20
seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR
15   protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W.
Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR
Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990,
Academic Press.

A suitable cDNA library from which a clone encoding canine
20   GHSR can be isolated from a cDNA library constructed from canine hypothalamic
poly (A)+ mRNA that has been cloned into a suitable vector, *e.g.*, the vector pcDNA-
3.1 (Invitrogen, San Diego, CA) by methods well-known in the art. The
primary clones of such a library can be subdivided into pools with each
pool containing approximately 20,000 clones and each pool can be
25   amplified separately.

By this method, a cDNA fragment encoding an open reading frame of
349 amino acids (SEQ.ID.NO.:2) can be obtained. This cDNA fragment can be
cloned into a suitable cloning vector or expression vector. For example, the fragment
can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San
30   Diego, Ca). Canine GHSR protein can then be produced by transferring an expression
vector encoding canine GHSR or portions thereof into a suitable host cell and growing
the host cell under appropriate conditions. Canine GHSR protein can then be isolated
by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone
35   encoding canine GHSR protein can be isolated from a cDNA library using as a probe

oligonucleotides specific for canine GHSR and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*, Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985,

5   *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for canine GHSR and that can be used to screen cDNA libraries can be readily designed based upon the cDNA sequence of canine GHSR shown in Figure 1 as SEQ.ID.NO.:1 and can be synthesized by methods well-known in the art.

10   Genomic clones containing the canine GHSR gene can be obtained from commercially available canine genomic libraries such as the canine lambda genomic DNA library from Stratagene (catalog number 946802). Alternatively, one may prepare canine genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the

15   canine GHSR can be isolated, using probes based upon the canine GHSR sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*, 1994, Nature Genet. 6:84-89).

The present invention also provides oligonucleotides, based upon SEQ.ID.NO.:1, that can be used to isolate the canine GHSR gene or

20   to isolate GHSR genes from other species. In particular, the present invention includes DNA oligonucleotides comprising at least 18 contiguous nucleotides of SEQ.ID.NO.:1. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotides can be packaged in kits.

25   The present invention makes possible the recombinant expression of the canine GHSR protein in various cell types. Such recombinant expression makes possible the study of this protein so that its biochemical mechanism of action and its physiological role can be elucidated. Accordingly, the present invention includes a method of expressing canine GHSR protein comprising:

30   (a)   transfecting host cells with an expression vector that encodes canine GHSR protein;

(b)   culturing the transfected cells of step (a) under conditions such that canine GHSR protein is expressed.

In particular embodiments, the cells are eukaryotic cells. In other embodiments, the cells are mammalian cells. In still other embodiments, the cells are COS cells, in particular COS-7 cells (ATCC CRL 1651), or melanophores.

The present invention also includes C-terminal truncated forms of

5   canine GHSR protein, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

It has been found that, in some cases, membrane spanning regions of

10   receptor proteins can be used to inhibit receptor function (Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227:200-204; Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Lofts *et al.*, Oncogene 8:2813-2820). Accordingly, the present invention provides peptides derived from the seven membrane spanning regions of canine GHSR protein and their use to inhibit canine GHSR function. Such peptides can

15   include the whole or parts of the receptor membrane spanning domains.

The present invention makes possible the development of assays which measure the biological activity of the canine GHSR protein. Such assays using recombinantly expressed canine GHSR protein are especially of interest. Assays for canine GHSR protein activity can be used

20   to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of canine GHSR protein. Such identified compounds can serve as "leads" for the development of veterinary pharmaceuticals that can be used to treat dogs having illnesses in which inappropriate activity of the canine GHSR protein

25   is involved. In versions of the above-described assays, mutant canine GHSR proteins are used and inhibitors or activators of the activity of the mutant canine GHSR proteins are discovered.

In general, such assays comprise:

(a)   recombinantly expressing canine GHSR protein or

30   mutant canine GHSR protein in a host cell;

(b)   measuring the biological activity of canine GHSR protein or mutant canine GHSR protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of canine GHSR protein or mutant canine GHSR protein;

where a change in the biological activity of the canine GHSR protein or the mutant canine GHSR protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of canine GHSR protein or mutant canine GHSR protein.

5   The biological activity of the canine GHSR protein can be measured by measuring various changes in the host cells, *e.g.*, an increase in intracellular calcium; depolarization and inhibition of potassium channels; increase in inositol triphosphate concentration; or an increase in the activity of protein kinase C (Howard et al., 1996, Science 273:974-977).

10   Using the cDNA provided by the present invention (SEQ.ID.NO.:1) and methods well-known in the art, complementary RNA (cRNA) can be prepared encoding the canine GHSR protein. This cRNA can be injected into *Xenopus* oocytes in order to cause the expression of canine GHSR protein in the oocytes. The oocytes can then be exposed to

15   substances suspected of being agonists of the canine GHSR protein. If the substance is actually an agonist, it will cause oscillatory increases in calcium activated chloride currents. In this manner, agonists of the canine GHSR can be identified. To improve the sensitivity of this assay, the oocytes can be injected with cRNA encoding the bioluminescent Ca^{2+}

20   sensitive protein aequorin and cRNA encoding a $G\alpha$ subunit (*e.g.*, $G\alpha11$) in addition to being injected with cRNA encoding canine GHSR protein. Such methods of assaying for agonists of other GPCRs are known in the art (see, *e.g.*, Howard et al., 1996, Science 273:974-977).

           The specificity of binding of compounds having affinity for canine

25   GHSR protein can be shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to canine GHSR or that inhibit the binding of a known, labeled ligand of canine GHSR to these cells, or membranes prepared from these cells, provides an effective method for the

30   rapid selection of compounds with high affinity for canine GHSR. The compounds need not necessarily be labeled but can be non-labeled compounds that can be used to displace bound labeled compounds or that can be used as activators in functional assays. Those compounds identified by the above method are likely to be agonists or

antagonists of canine GHSR and may be peptides, proteins, or non-proteinaceous organic molecules.

Therefore, the present invention includes assays by which canine GHSR agonists and antagonists may be identified. Analogous methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of canine GHSR. For example, Cascieri *et al.*, 1992, Molec. Pharmacol. 41:1096-1099 describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor.

Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of canine GHSR that comprises:

(a)   transfecting cells with an expression vector encoding canine GHSR;

(b)   allowing the transfected cells to grow for a time sufficient to allow canine GHSR to be expressed;

(c)   harvesting the transfected cells and resuspending the cells in the presence of a known labeled agonist of the canine GHSR in the presence and in the absence of the substance;

(d)   measuring the binding of the labeled agonist to canine GHSR; where if the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of canine GHSR.

In a modification of the above-described method, step (b) is modified in that the cells are stably transfected with the expression vector containing canine GHSR. In another modification of the above-described method, step (c) is modified in that the cells are not harvested and

5   resuspended but rather the radioactively labeled known agonist and the substance are contacted with the cells while the cells are attached to a substratum, *e.g.*, tissue culture plates.

The conditions under which step (c) of the above-described method is practiced are conditions that are typically used in the art for the

10   study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

The present invention also includes a method for determining whether a substance is capable of binding to canine GHSR, *i.e.*, whether

15   the substance is a potential agonist or an antagonist of canine GHSR, where the method comprises:

(a)   providing test cells by transfecting cells with an expression vector that directs the expression of canine GHSR in the cells;

(b)   exposing the test cells to the substance;

20   (c)   measuring the amount of binding of the substance to canine GHSR in the test cells;

(d)   comparing the amount of binding of the substance to canine GHSR in the test cells with the amount of binding of the substance to the same cells that have not been transfected with canine GHSR.

25   Where the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to canine GHSR and thus is a likely agonist or antagonist of canine GHSR. Determining whether the substance is actually an agonist or antagonist can then be accomplished by the use of functional assays

30   such as, *e.g.*, the *Xenopus* oocyte assay described above and in Howard et al., 1996, Science 273:974-977 or the assay involving the use of promiscuous G-proteins described below.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of

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protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In an modification of the above method, the present invention
5   includes a method for determining whether a substance is capable of binding to canine GHSR comprising:

(a)   providing test cells by transfecting cells with an expression vector that directs the expression of canine GHSR in the cells;

(b)   exposing the test cells to a known ligand of canine
10   GHSR under conditions such that the known ligand of canine GHSR binds to the canine GHSR in the test cells;

(c)   subsequently or concurrently to step (b), exposing the test cells to a substance that is suspected of being capable of binding to canine GHSR;

15   (d)   measuring the amount of binding of the known ligand of canine GHSR to canine GHSR in the presence and the absence of the substance;

(e)   comparing the amount of binding of the known ligand of canine GHSR to canine GHSR in the presence and the absence of the
20   substance where a decrease in the amount of binding of the known ligand of canine GHSR to canine GHSR in the presence of the substance indicates that the substance is capable of binding to canine GHSR.

The assays described above can be carried out with cells that have been transiently or stably transfected with canine GHSR.
25   Transfection is meant to include any method known in the art for introducing canine GHSR into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing canine GHSR, and electroporation.

30   Where binding of the substance or agonist to canine GHSR is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, *e.g.*, radioactively, fluorescently, enzymatically. Similarly, binding of a known ligand of canine GHSR to

canine GHSR can be measured by employing a known ligand of canine GHSR that has been labeled. Known ligands of canine GHSR can be chosen from among the group of known ligands for human, rat, or swine GHSRs.

The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells and those membranes can be exposed to the substance. Such a modification utilizing membranes rather than cells is well known in the art and is described in, *e.g.*, Hess *et al.*, 1992, Biochem. Biophys. Res. Comm. 184:260-268.

In particular embodiments of the above-described methods, canine GHSR has the amino acid sequence shown in SEQ.ID.NO.:2.

In particular embodiments of the above-described methods, the expression vector comprises SEQ.ID.NO.:1.

In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

The present invention includes assays by which canine GHSR agonists and antagonists may be identified by their ability to stimulate or antagonize a functional response mediated by canine GHSR. Canine GHSR belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the Gα subunit of the G-protein to disassociate from the Gβ and Gγ subunits. The Gα subunit (and sometimes the Gβ and Gγ subunits) can then go on to activate a variety of second messenger systems.

There are many types of G-proteins, with many types of subunits. Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR,

it is usually necessary to ensure that the proper G-protein is present in
the system containing the GPCR. It has been found, however, that there
are certain G-proteins that are "promiscuous." These promiscuous G-
proteins will couple to, and thus transduce a functional signal from,

5   virtually any GPCR. See Offermanns & Simon, 1995, J. Biol. Chem.
270:15175, 15180 (Offermanns). Offermanns described a system in which
cells are transfected with expression vectors that result in the expression
of one of a large number of GPCRs as well as the expression of one of the
promiscuous G-proteins Gα15 or Gα16. Upon the addition of an agonist of

10   the GPCR to the transfected cells, the GPCR was activated and was able,
via Gα15 or Gα16, to activate the β isoform of phospholipase C, leading to
an increase in inositol phosphate levels in the cells.

                Since canine GHSR functions in the aequorin assay, it is
likely to be coupled through Gα q11. Thus, one could set up functional assays for

15   canine GHSR by transfecting cells with an expression vector encoding SEQ.ID.NO.:1
as well as an expression vector encoding Gα q11. Alternatively, by making use
of these promiscuous G-proteins as in Offermanns, it is possible to set up
functional assays for canine GHSR, even in the absence of knowledge of
the G-protein with which canine GHSR is coupled *in vivo*. One possibility

20   is to create a fusion or chimeric protein composed of the extracellular and
membrane spanning portion of canine GHSR fused to a promiscuous G-
protein. Such a fusion protein would be expected to transduce a signal
following binding of ligand to the canine GHSR portion of the fusion
protein. Accordingly, the present invention provides a method of

25   identifying antagonists of canine GHSR comprising:

                (a)   providing cells that expresses a chimeric canine GHSR
protein fused at its C-terminus to a promiscuous G-protein;

                (b)   exposing the cells to an agonist of canine GHSR;

                (c)   subsequently or concurrently to step (b), exposing the

30   cells to a substance that is a suspected antagonist of canine GHSR;

                (d)   measuring the level of inositol phosphates in the cells;

                where a decrease in the level of inositol phosphates in the cells in the
presence of the substance as compared to the level of inositol phosphates in the cells

- 16 -

in the absence of the substance indicates that the substance is an antagonist of canine GHSR.

Another possibility for utilizing promiscuous G-proteins in connection with canine GHSR includes a method of identifying agonists of canine GHSR comprising:

(a)   providing cells that expresses both canine GHSR and a promiscuous G-protein;

(b)   exposing the cells to a substance that is a suspected agonist of canine GHSR;

(c)   measuring the level of inositol phosphates in the cells;

where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of canine GHSR.

Another possibility for utilizing promiscuous G-proteins in connection with canine GHSR includes a method of identifying agonists of canine GHSR comprising:

(a)   providing test cells that expresses both canine GHSR and a promiscuous G-protein;

(b)   providing control cells that are identical to the cells of step (a) except that the control cells do not express canine GHSR;

(c)   exposing the test cells and the control cells to a substance that is a suspected agonist of canine GHSR;

(c)   measuring the level of inositol phosphates in the test cells and the control cells;

where an increase in the level of inositol phosphates in the test cells as compared to the level of inositol phosphates in the control cells indicates that the substance is an agonist of canine GHSR.

Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻)

(ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL

5   1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

In a particular embodiment of the above-described methods, the cells are transfected with expression vectors that direct the expression of canine GHSR and the promiscuous G-protein in the cells.

The conditions under the cells are exposed to the substance

10   are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described methods,

15   the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16. Expression vectors containing Gα15 or Gα16 are known in the art. See, *e.g.*, Offermanns; Buhl *et al.*, 1993, FEBS Lett. 323:132-134; Amatruda *et al.*, 1993, J. Biol. Chem. 268:10139-10144.

The above-described assay can be easily modified to form a

20   method to identify antagonists of canine GHSR. Such a method is also part of the present invention and comprises:

(a)   providing cells that expresses both canine GHSR and a promiscuous G-protein;

(b)   exposing the cells to a substance that is an agonist of

25   canine GHSR;

(c)   subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of canine GHSR;

(d)   measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the

30   cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of canine GHSR.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are

mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻)
(ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji
(ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7
(ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92),

5   NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL
1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The conditions under which steps (b) and (c) of the method
are practiced are conditions that are typically used in the art for the study
of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such

10   as those represented by such commonly used buffers as PBS or in tissue
culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method,
the cells are transfected with expression vectors that direct the expression
of canine GHSR and the promiscuous G-protein in the cells.

15   In a particular embodiment of the above-described method,
the promiscuous G-protein is selected from the group consisting of Gα15 or
Gα16.

In particular embodiments of the above-described methods, canine
GHSR has the amino acid shown in SEQ.ID.NO.:2.

20   While the above-described methods are explicitly directed to
testing whether "a" substance is an activator or an inhibitor of canine
GHSR, it will be clear to one skilled in the art that such methods can be
adapted to test collections of substances, *e.g.*, combinatorial libraries, to
determine whether any members of such collections are activators or

25   inhibitors of canine GHSR. Accordingly, the use of collections of
substances as the substance in the above-described methods is within the
scope of the present invention.

Given the wide range of utility displayed by known agonists
and antagonists of human GHSRs, it is clear that those skilled in the art

30   would consider the agonists and antagonists of canine GHSR identified by
the methods of the present invention to be pharamacologically useful in a
similar manner in canines. In addition to their use in canines, agonists
and antagonists of canine GHSR identified by the above-described
methods will be useful for guiding the choice of compounds with ideal

pharmaceutical properties (e.g. oral bioavailability, duration of action) to be used in humans or other animal species (e.g. swine) for growth promoting effects.

When screening compounds in order to identify potential
5   pharmaceuticals that specifically interact with a target receptor, it is necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find compounds that are
10   potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus target") and produce the desired pharmacological effect through receptor A, it is also necessary to determine that the compounds do not interact with receptors B, C, D, etc. (the "minus targets"). In general, as part of a
15   screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). Canine GHSR protein and DNA encoding canine GHSR protein have utility in that they can be used as "minus targets" in screens design to identify compounds that specifically interact with other G-protein coupled
20   receptors.

The present invention also includes antibodies to the canine GHSR protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire canine GHSR protein or against suitable antigenic
25   fragments of the protein that are coupled to suitable carriers, e.g., serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, e.g., Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer
30   Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, canine GHSR protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, e.g., rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of

antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, canine GHSR protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate

5   non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow

10   & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

The following non-limiting examples are presented to better illustrate the invention.

                                    EXAMPLE 1

15   Isolation of full-length genomic clones for canine GHSR

A commercially available canine spleen genomic library in lambda dash vector (Stratagene, La Jolla, CA) was employed for cloning of a dog GHSR type 1a and can be used to isolate other canine GHSR family members. Approximately 1.8×10^6 clones were plated and the plaques were then lifted onto

20   Nytran extra strength filters (Schleicher & Schuell, Keene, NH) according to the manufacturer's specifications. The filters were pre-hybridized in 50% formamide, 5X SSPE, 2X Denhardt's, 0.1% SDS, 100 μg/ml salmon sperm DNA for 2 hrs at 30°C followed by overnight hybridization at 30°C in 50% formamide, 5XSSPE, 2X Denhardt's, 0.1% SDS, 10% dextran sulfate, 100 μg/ml salmom sperm DNA. To

25   identify positive clones, the open reading frame of human type 1a GHSR (1.1 kb) was random primed using Strategene Prime It II kit and added to hybridization solution at 1×10^6 cpm/ml. Posthybridization, the filters were washed in moderate stringency (1X SSC, 0.1% SDS at 55°C for 30 min) and exposed to X-ray film. Three rounds of plaque purification revealed 5 positive clones (3, 19, 25, 27 and 28). Lambda phage

30   liquid lysate preparation was performed according to a protocol supplied by Clontech labs. This DNA was digested with Sal I to generate 22 kb and 9 kb DNA fragments. For clone 19, the 22 kb insert which hybridized to human type 1a probe was subcloned into pGem 11 zf+ Sal I site (Promega). Colony hybridization of

approximately 1,000 transformants uncovered a single hybridizing subclone which was sequenced using the ABI prism FS dye terminator sequencing kit (Applied Biosystems, Foster City, CA). The other four clones were sequenced directly from the lambda DNA using newly available ABI prism FS bigdye terminator cycle

5   sequencing kit.

EXAMPLE 2

Cloning of canine cDNA for the GHSR

Dog hypothalamus mRNA was prepared from 1 g dog hypothalamus

10   using the Poly(A) Pure mRNA isolation kit (Ambion Inc, Austin, TX) according to the manufacturer's instructions. cDNA was prepared from the mRNA using the Choice Superscript cDNA kit (BRL, Bethesda, MD). After the column chromatography step, the cDNA was quantitated using DNA Dipstick (Invitrogen, CA). 10 ng cDNA was used as template for PCR using dog specific primers PCR1S

15   (GGGCCC GAATTC GCC GCC ATG CGG AAC GCG ACG GCC CGC (SEQ.ID.NO.:8)) and PCR2AS (AGTTTA GCGG CCGC TCA TGT ATT AAT ACT AGA CTC TGT (SEQ.ID.NO.:9)). PCR reactions comprised 10 mM Tris (pH 8.0), 50 mM KCl, 1.5 mM MgCl2, 100 µg/ml gelatin , 250 µM each dNTP, and 10% DMSO with 20 pmoles of each primer and 20 ng of target DNA. Prior to the addition

20   of Taq polymerase (Pharmacia), the reactions were heated at 100°C for 10 minutes followed by soaking at 72°C. Taq polymerase was added to a final concentration of 0.25 units/ml. The cycling parameters were 94°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute, for a total of 30 cycles, followed by incubation at 72°C for 10 minutes. Reactions were electrophoresed on a 1% Seakem GTG agarose gel in 1X

25   TAE buffer and visualized under long wave UV light. The fragment of expected size (1.2 kb) for the ORF (open reading frame) was electroeluted and TA cloned (Invitrogen, CA), sequenced with dye terminator cycle sequencing ready reactions (Perkin Elmer, Foster City, CA), and analyzed on a 377 ABI Prism cycle sequencer. The fragment was subcloned into the pCDNA3 vector for expression. Overall, the

30   amino acid sequence (Figure 3) of the cloned canine GHSR is 91% identical to the rat GHSR and 89% identical to the human GHSR. The most divergent feature of the canine GHSR compared to GHSR from other species is the absence of 17 amino acids in the N-terminal extracellular domain.

EXAMPLE 3

Radiolabeled MK-677 Binding Assay

5   Expression of the canine GHSR was monitored using a [^{35}S]-MK-677 binding assay conducted on cells transfected with a canine GHSR expression plasmid (full-length open reading frame placed in the mammalian expression vector pcDNA-3). Mammalian cells (COS-7, HEK-293) were transfected with GHSR expression plasmids using Lipofectamine (GIBCO-BRL; Hawley-Nelson, 1993, Focus 15:73). Transfections were performed in 60 mm dishes on 80% confluent cells

10   (approximately 4 x 10^{5}cells) with 8 μg of Lipofectamine and 32 μg of GHSR plasmid DNA.

       Binding of ^{35}S- MK-677 to crude membranes prepared from COS-7 or HEK-293 cells transfected with GHSR expression plasmids was conducted. Crude cell membranes from COS-7 transfectants were prepared on ice, 48 hrs post-

15   transfection. Each 60 mm dish was washed twice with 3 ml of PBS, once with 1 ml homogenization buffer (50 mM Tris-HCl (pH 7.4), 5 mM MgCl$_2$, 2.5 mM EDTA, 30 μg/ml bacitracin). 0.5 ml of homogenization buffer was added to each dish, cells were removed by scraping and then homogenized using a Polytron device (Brinkmann, Syosset, NY; 3 bursts of 10 seconds at setting 4). The homogenate was

20   then centrifuged for 20 minutes at 11,000 x g at 0°C and the resulting crude membrane pellet (chiefly containing cell membranes and nuclei) was resuspended in homogenization buffer supplemented with 0.06% BSA (0.1 ml/60 mm dish) and kept on ice. Binding reactions were performed at 20°C for 1 hr in a total volume of 0.5 ml containing: 0.1 ml of membrane suspension, 10 μl of ^{35}S-MK-677 (0.05 to 1 nM;

25   specific activity approximately 900 Ci/mmol), 10 μl of competing drug, and 380-390 μl of homogenization buffer. Bound radioligand was separated by rapid vacuum filtration (Brandel 48-well cell harvester) through GF/C filters pretreated for 1 hr. with 0.5% polyethylenimine. After application of the membrane suspension to the filter, the filters were washed 3 times with 3 ml each of ice cold 50 mM Tris-HCl (pH

30   7.4), 10 mM MgCl$_2$, 2.5 mM EDTA, and 0.015% Triton X-100, and the bound radioactivity on the filters was quantitated by scintillation counting. Specific binding (> 90% of total) is defined as the difference between total binding and non-specific

binding conducted in the presence of 50 nM unlabeled MK-677. Competition studies showed that the cloned canine GHSR binds MK-677 with an IC_{50} value of 2.2 nM, which compares favorably to the native canine pituitary GHSR (1 nM) and cloned human GHSR (0.8 nM).

5

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the

10   scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1.   A recombinant DNA molecule encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:2.

2.   The recombinant DNA molecule of claim 1 comprising the nucleotide sequence SEQ.ID.NO.:1.

3.   A DNA molecule that hybridizes under stringent conditions to SEQ.ID.NO.:1.

4.   An expression vector comprising the DNA of claim 1.

5.   A recombinant host cell comprising the expression vector of claim 4.

6.   A canine GHSR protein, substantially free from other proteins, having the amino acid sequence SEQ.ID.NO.:2.

7.   The protein of claim 6 containing a single amino acid substitution.

8.   The protein of claim 7 where the substitution is a conservative substitution.

9.   The protein of claim 6 containing two or more amino acid substitutions where the substitutions do not occur in positions where all four of the human, rat, swine, and canine GHSRs share the same amino acid and where the protein has substantially the same biological activity as canine GHSR having the amino acid sequence SEQ.ID.NO.:2.

10.   A method of expressing canine GHSR protein comprising:

(a)   transfecting host cells with an expression vector that encodes canine GHSR protein having the amino acid sequence SEQ.ID.NO.:2;

(b)   culturing the transfected cells of step (a) under conditions such that canine GHSR protein is expressed.

11.   A method for identifying an activator or an inhibitor of
5   canine GHSR protein, comprising:

(a)   recombinantly expressing canine GHSR protein having the amino acid sequence SEQ.ID.NO.:2 in a host cell;

(b)   measuring the biological activity of canine GHSR protein in the host cell in the presence and in the absence of a substance
10   suspected of being an activator or an inhibitor of canine GHSR protein;

where a change in the biological activity of the canine GHSR protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of canine GHSR protein.

15

12.   A method for determining whether a substance is a potential agonist or antagonist of canine GHSR that comprises:

(a)   transfecting cells with an expression vector encoding canine GHSR protein having the amino acid sequence SEQ.ID.NO.:2;

20   (b)   allowing the transfected cells to grow for a time sufficient to allow canine GHSR protein to be expressed;

(c)   exposing the transfected cells to a known labeled agonist of the canine GHSR in the presence and in the absence of the substance;

25   (d)   measuring the binding of the labeled agonist to canine GHSR or measuring the functional response of canine GHSR to the agonist;

where if the amount of binding of the known agonist to canine GHSR or the functional response of canine GHSR to the agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential
30   agonist or antagonist of canine GHSR.

ATGCGGAACGCGACGCCCGAGGGCCCGGGTCGGCGGGGCTCGGGACCTGCTGCTGCGGCTGCGGCTGTTCCCCGCG
CCGCTGCTGCGGCGGCCTGACGGCGCCACCTGCTGCGTGGCGGCTGTTCGCGCTGGGCGTGCGGGGCGTGCGGGGCCAACCTGCTG
ACGGTGCTGGTGCGGGCGCTTCCCGCGAGCTGCCGCACCACCAACCTGTACCTGTGCAGCCTGCCC
TGCTCCGACCTGCTCATCTTCCTGTCGCATGCCGCCTCGACCTGGTGCGCCTGTGCCAGTACCGGCCCTGG
ACCTTCGGCGACCTGCTCTGCAAACTCTTCCAGTTCGTGAGCGAGGGCTGCACCTACGCCACGGTGCTC
ACCATCACGGGCGCTGAGCGTCGAGCGGCTACTTCGGCCATCTGCTTCCCGCGTGCGCGCCAAGGTGCTGGTG
ACCAAGGGCGCGTGAAGCTGGCCCTGCTGCGCCATCTGGGCCGTGGCCTTCTGCAGCGCCGGGCCCACC
TTCGTGCTGGTGGCGGTGGAGCAGGAGAACGGCACCCGCACCCCCGGACACCCCGGAGTGCCGCGCCACC
GAGTTCGCCGTGCTGCGGGCGGTGCTCTCACGGCCCATGGTGGGTGTCCAGCGTCTTCTTCTTCCTGCCC
GTCTTCTGCCTCACGGTGCTCTACGGCCTCATCGGCGAGCAGGCCCGCCGGAAGATGCCGTGGCGTGTTT
GCGGGGGGCCTCGCTCCGGAGCAGAGAGCCACCGGGTGGGGCGGATATTTATTTCCAAGTCCTTCGAGCCCGGC
TCCTTGGAGATTGCTCAGATCAGCCAATACTGCAACCTGGTATCCTTTGTCCTCTTCTACCTCAGTGCT
GCCATCAATCCCATTCGTACAACATCATGTCCAAGAAGTACCGGGTGGGGTGTTCAAGCTTCTGGGA
TTTGAACCCTTCTCCCAGAGGAAGCTCTCCACTCTGAAGGATGAAAGTTCTCGGGCCTCTGGACAGAGTCT
AGTATTAATACATGA (SEQ. ID. NO.: 1)

FIG.1

MRNATAREGPGSAGWDLLPLFPAPLLAGVTATCVALFAVGVAGNLLTVLVVRRFRELRTTTN
LYLCSLACSDLLIFLCMPLDLVRLWQYRPWTFGDLLCKLFQFVSEGCTYATVLTITALSVERYF
AICFPLRAKVLVTKGRVKLALLAIWAVAFCSAGPIFVLVGVEHENGTDPRDTRECRATEFAVRS
GLLTAMWWSSVFFFLPVFCLTVLYGLIGRKLWRRGRGDTAGGASLREQSHRQTVKMLAVVV
FAFIFCWLPFHVGRYLFSKSFEPGSLEIAQISQYCNLVSFVLFYLSAAINPILYNIMSKKYRVAVF
KLLGFEPFSQRKLSTLKDESSRAWTESSINT (SEQ.ID.NO.:2)

FIG.2

huGHS-R   1 MWNATPSEEPGFNLTLADLDWDASPGNDSLGDEL 34
ratGHS-R   1 MWNATPSEEPEPNVTL-DLDWDASPGNDSLPDEL 33
swGHS-R   1 MWNATPSEEPGPNLTLPDLGWDAPPENDSLVEEL 34
dogGHS-R   1 MRNATAREGPGSA------GWD-----------L 17

huGHS-R   35 LQLFPAPLLAGVTATCVALFVVGIAGNLLTMLVV 68
ratGHS-R 34 LPLFPAPLLAGVTATCVALFVVGISGNLLTMLVV 67
swGHS-R   35 LPLFPIPLLAGVTATCVALFVVGIAGNLLTMLVV 68
dogGHS-R 18 LPLFPAPLLAGVTATCVALFAVGMAGNLLTMLVV 51

huGHS-R   69 SRFRELRTTTNLYLSSMAFSDLLIFLCMPLDLVR 102
ratGHS-R 68 SRFRELRTTTNLYLSSMAFSDLLIFLCMPLDLVR 101
swGHS-R   69 SRFREMRTTTNLYLSSMAFSDLLIFLCMPLDLFR 102
dogGHS-R 52 RRFRELRTTTNLYLCSLACSDLLIFLCMPLDLVR 85

huGHS-R 103 LWQYRPWNFGDLLCKLFQFVSESCTYATVLTITA 136
ratGHS-R 102 LWQYRPWNFGDLLCKLFQFVSESCTYATVLTITA 135
swGHS-R 103 LWQYRPWNLGNLLCKLFQFVSESCTYATVLTITA 136
dogGHS-R 86 LWQYRPWTFGDLLCKLFQFVSEGCTYATVLTITA 119

huGHS-R 137 LSVERYFAICFPLRAKVVVTKGRVKLVIFVIWAV 170
ratGHS-R 136 LSVERYFAICFPLRAKVVVTKGRVKLVILVIWAV 169
swGHS-R 137 LSVERYFAICFPLRAKVVVTKGRVKLVILVIWAV 170
dogGHS-R 120 LSVERYFAICFPLRAKVLVTKGRVKLALLAIWAV 153

huGHS-R 171 AFCSAGPIFVLVGVEHENGTDPWDTNECRPTEFA 204
ratGHS-R 170 AFCSAGPIFVLVGVEHENGTDPRDTNECRATEFA 203
swGHS-R 171 AFCSAGPIFVLVGVEHDNGTDPRDTNECRATEFA 204
dogGHS-R 154 AFCSAGPIFVLVGVEHENGTDPRDTRECRATEFA 187

FIG. 3A

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huGHS-R   205  VRSGLLTVMVWVSSIFFFLPVFCLTVLYSLIGRK  238
ratGHS-R  204  VRSGLLTVMVWVSSVFFFLPVFCLTVLYSLIGRK  237
swGHS-R   205  VRSGLLTVMVWVSSVFFFLPVFCLTVLYSLIGRK  238
dogGHS-R  188  VRSGLLTAMVWVSSVFFFLPVFCLTVLYGLIGRK  221


huGHS-R   239  LWRRRRGDAVVGASLRDQNHKQTVKMLAVVVFAF  272
ratGHS-R  238  LWRRR-GDAAVGASLRDQNHKQTVKMLAVVVFAF  270
swGHS-R   239  LWRRKRGEAAVGSSLRDQNHKQTVKMLAVVVFAF  272
dogGHS-R  222  LWRRGRGDTAGGASLRELQSHRQTVKMLAVVVFAF 255


huGHS-R   273  ILCWLPFHVGRYLFSKSFEPGSLEIAQISQYCNL  308
ratGHS-R  271  ILCWLPFHVGRYLFSKSFEPGSLEIAQISQYCNL  304
swGHS-R   273  ILCWLPFHVGRYLFSKSLEPGSVEIAQISQYCNL  306
dogGHS-R  256  IFCWLPFHVGRYLFSKSFEPGSLEIAQISQYCNL  289


huGHS-R   307  VSFVLFYLSAAINPILYNIMSKKYRVAVFRLLGF  340
ratGHS-R  305  VSFVLFYLSAAINPILYNIMSKKYRVAVFKLLGF  338
swGHS-R   307  VSFVLFYLSAAINPILYNIMSKKYRVAVFKLLGF  340
dogGHS-R  290  VSFVLFYLSAAINPILYNIMSKKYRVAVFKLLGF  323


huGHS-R   341  EPFSQRKLSTLKDESSRAWTESSINT          366
ratGHS-R  339  ESFSQRKLSTLKDESSRAWTKSSINT          364
swGHS-R   341  EPFSQRKLSTLKDESSRAWTESSINT          366
dogGHS-R  324  EPFSQRKLSTLKDESSRAWTESSINT          349
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FIG. 3B